

Song System Auditory Responses Are Stable and Highly Tuned During Sedation, Rapidly Modulated and Unselective During Wakefulness, and Suppressed By Arousal

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Cardin, Jessica A. and Marc F. Schmidt. Song system auditory responses are stable and highly tuned during sedation, rapidly modulated and unselective during wakefulness, and suppressed by arousal. *J Neurophysiol* 90: 2884–2899, 2003. First published July 23, 2003; 10.1152/jn.00391.2003. We used auditory responsiveness in the avian song system to investigate the complex relationship between behavioral state and sensory processing in a high-order sensorimotor brain area. We present evidence from recordings in awake, anesthetized, and sleeping male zebra finches (*Taeniopygia guttata*) that auditory responsiveness in nucleus HVC is profoundly affected by changes in behavioral state. In anesthetized and sleeping birds, auditory responses were characterized by an increase in firing rate that was selective for the bird's own song (BOS) and highly stable over time. In contrast, HVC responses during wakefulness were extremely variable and transitioned between undetectable and robust levels over short intervals. Surprisingly, auditory responses in awake birds were not selective for the BOS stimulus. The variability of HVC auditory responses in awake birds suggests that, as in mammals, wakefulness is not a uniform behavioral state. Rather, auditory responsiveness likely is continually influenced by variables such as arousal state. We therefore developed several experimental paradigms in which we could manipulate arousal levels during auditory stimulus presentation. In all cases, arousal suppressed HVC auditory responses. This effect was specific to the song system, as auditory responses in Field L, a primary auditory area that is a source of auditory input to HVC, were unaffected. While arousal acts as a negative regulator of HVC auditory responsiveness, the presence and variability of the responses observed in awake, alert birds suggests that other mechanisms, such as attention, may enhance auditory responsiveness. The interplay between behavioral state and sensory processing may regulate song system responsiveness according to the bird's behavioral and social context.

INTRODUCTION

The concept of behavioral state is a composite representation of the neural, endocrine, cardiovascular, and muscular activity of an animal. Frequently studied behavioral states include sedation, anesthesia, sleep, and both restrained and freely moving wakefulness. Brain functions, particularly sensory processing, are profoundly affected by behavioral state. For example, the mammalian thalamus accurately relays sensory inputs to the cortex while in the activated waking state but not during the burst-firing mode of slow-wave sleep and anesthesia (Castro-Alamancos 2002a,b; Poggio and Mountcastle 1963; Weyand et

al. 2001). Not surprisingly, thalamic and cortical neurons respond differently to sensory stimuli during wakefulness versus sleep or anesthesia (Coenen and Vendrik 1972; Gucer 1979; Livingstone and Hubel 1981; Steriade et al. 1969; Swadlow and Weyand 1987). Similarly, several studies have indicated that neurons in the avian song system respond differently to auditory stimuli during wakefulness and sleep or anesthesia (Dave et al. 1998; Nick and Konishi 2001; Rauske et al. 2003; Schmidt and Konishi 1998). Because songbirds produce highly stereotyped vocal motor behaviors that are modulated by social context (Jarvis et al. 1998), the song system provides an excellent model in which to explore behavioral state-dependent modulation of sensory processing.

The avian song system is composed of discrete, interconnected nuclei dedicated to the learning, production, and perception of bird song. The song system includes two main pathways: the motor pathway, which is necessary for song motor output, and the anterior forebrain pathway, which is essential for song learning and maintenance (Fig. 1). Nucleus HVC, a sensorimotor area that is part of both pathways, receives auditory afferents from nucleus interfascialis (NIf), a major site of auditory input to the song system (Janata and Margoliash 1999), and provides auditory input to RA and the anterior forebrain pathway (Doupe and Konishi 1991; Vicario and Yohay 1993).

Several lines of evidence suggest that HVC plays a role in auditory processing. HVC neurons respond preferentially to the bird's own song (BOS) over other auditory stimuli during sleep and anesthesia (Dave and Margoliash 2000; Margoliash 1983, 1986; Margoliash and Fortune 1992; Mooney 2000; Theunissen and Doupe 1998). Auditory feedback to the song system, most likely via HVC, is essential for both juvenile song learning and adult song maintenance. In addition, lesions to HVC impair song perception and discrimination by adult birds (Brenowitz 1991; Gentner et al. 2000; Okanoya and Watanabe 1995). Given these findings, HVC neurons might be expected to respond strongly to auditory stimuli during wakefulness.

Surprisingly, previous work has suggested that HVC responds weakly or not at all to auditory stimuli during wakefulness (Nick and Konishi 2001; Schmidt and Konishi 1998). In contrast, other work has indicated that there may be conditions under which some HVC neurons demonstrate auditory

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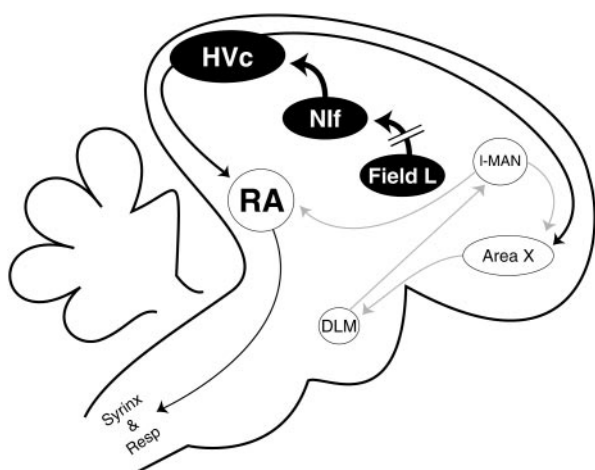


FIG. 1. Schematic of the avian song system. HVC (used as proper name), which is part of both the anterior forebrain pathway and the vocal motor pathway, receives auditory input indirectly from Field L via nucleus interfascialis (Nif). The anterior forebrain pathway includes HVC, song-related region of the basal ganglia (Area X), medial portion of the dorsal lateral nucleus of the anterior thalamus (DLM), lateral portion of the magnocellular nucleus of the anterior neostriatum (IMAN), and robust nucleus of the archistriatum (RA). The motor pathway includes Nif, HVC, and RA.

responses during wakefulness (Dave et al. 1998; Rauske et al. 2003). These studies have focused on large-scale changes in behavioral state, such as from sleep or anesthesia to wakefulness. However, more subtle changes within a general behavioral state, such as changes in arousal level during wakefulness, may be more relevant to normal song system function in awake birds and may explain some apparently contradictory findings from previous studies (Dave et al. 1998; Nick and Konishi 2001; Rauske et al. 2003; Schmidt and Konishi 1998). In addition, little is known about the specific aspects of changes in behavioral state that directly affect song-system auditory responsiveness. Thus the purpose of this study was to more fully explore the relationship between behavioral state and auditory responses in HVC.

We investigated the modulation of HVC auditory responses during both chronic recordings in freely behaving birds and acute recordings where behavioral state was carefully controlled. Here we show that HVC demonstrates robust, unselective auditory responses during wakefulness and that these responses are modulated over short time periods. We also show suppression of HVC auditory responsiveness by arousal from sedation and sleep. Some of these data have been presented previously in abstract form (Cardin and Schmidt 2002; Cardin et al. 2001).

METHODS

Animals

Adult male zebra finches (*Taeniopygia guttata*) ranging from 120 to 500 days of age were obtained from our breeding colony and from a local supplier. Birds were housed under constant 12:12 light:dark conditions and given food and water *ad libitum*. All procedures described here were approved by an institutional animal care and use committee at the University of Pennsylvania.

Auditory stimuli

The song of each bird was recorded several days prior to surgeries or experiments on that bird. Songs were recorded in a sound attenu-

ation chamber and digitized at 40 kHz with Goldwave (Goldwave, St. Johns, NF, Canada). For BOS stimuli, two song motifs were presented in normal orientation so that the bird heard the motifs as he would when singing. For reversed-BOS stimuli (REV), a two-motif segment was played backward. Most auditory stimuli were presented at 70 dB sound pressure level (SPL) peak intensity. For some acute recording experiments, auditory stimulus intensity was lowered after observing during initial tests for auditory responsiveness that the stimulus alone elicited behavioral indicators of arousal.

Chronic recordings

Electrodes and equipment were as described in Schmidt and Konishi (1998). The bird was anesthetized with 0.07 ml ketamine/xylazine (40 mg/kg ketamine and 8 mg/kg xylazine, Phoenix Pharmaceuticals, St. Joseph, MO). Small incisions were made in the scalp and skull, and nichrome wire (37.5 μ m diam; A-M Systems, Everett, WA) electrodes (0.2–0.7 M Ω) were implanted in HVC. Electrodes were then secured in place with dental cement and attached to a connector (Omnetics Connector Corporation, Minneapolis, MN) on the skull. A silver ground wire was inserted under the skull and cemented in place. Adult zebra finches have a two-layered skull with bony spicules separating the layers. Because dental cement usually extends between the layers, chronic implants of this sort are extremely stable (Dave et al. 1999).

Birds were allowed to recover for several days after surgery before being placed in a recording chamber and connected to a lightweight operational amplifier (Texas Instruments, Dallas, TX). The amplifier was connected to a mercury commutator that allowed each bird a full range of movement. Neural signals were sent through an HS4 headstage to a DB4 bioAmp controller and an FT6–2 anti-aliasing filter (Tucker Davis Technologies, Gainesville, FL) and digitized at 20 kHz with a 100-MHz, 16-bit DAQ board (National Instruments, Austin, TX). The birds remained connected for 3–5 days at a time and were kept on a consistent light cycle throughout the experiments. The birds did not appear stressed by the recording chamber, as they sang regularly. They were handled and disturbed as little as possible to observe normal variations in behavior and arousal state. Birds had access to food and water *ad libitum*.

During awake auditory trials, the birds were continuously observed on a video monitor to ensure that their eyes were open and that they were moving in the chamber. All awake auditory trials were conducted with the lights on. Any trials on which a bird did any of the following were discarded: vocalized, closed its eyes for two or more consecutive seconds, tucked its head under a wing, or crouched on the floor of the cage without moving. Auditory experiments during wakefulness consisted of 30–60 auditory stimulus trials of 6 s each. During each trial, 2 s of baseline recording was followed by $\sim$ 2 s of auditory stimulation and two additional seconds of baseline. The time interval between trials was varied during each experiment and ranged from 5 s to 3 min.

For arousal experiments, the implanted birds were given an intramuscular injection of the GABA_A receptor agonist diazepam (Abbott Laboratories, North Chicago, IL) (Kleingoor et al. 1993; Rudolph et al. 1999). This dose of diazepam resulted in a state of mild sedation. Diazepam was chosen because the birds were easily aroused and would then immediately return to a resting state within a few seconds (see RESULTS) and, as under other anesthetics, HVC auditory responses under diazepam are highly selective for the BOS over other auditory stimuli. Birds were allowed to rest 15–20 min between the injection and the auditory presentations. Arousal experiments consisted of a block of 30–60 BOS stimuli with each trial lasting 6 s. The time interval between trials ranged from 5 s to 3 min. At the beginning of randomly selected trials, the bird was aroused by lightly touching a padded tool to the chest feathers. Birds were considered aroused when their eyes were open and they moved about the cage during the auditory stimulus. Birds usually remained aroused and moving for

5–15 s. Arousal trials on which the bird did not open its eyes and move were discarded without looking at the recorded data. Likewise, resting trials on which the bird opened its eyes or moved were also discarded.

For sleep trials, the light was turned off and the bird allowed to rest in the dark until asleep. Usually, this took ~30 min. Birds were continuously observed via an infrared camera and video monitor and defined as asleep when they assumed a typical sleep posture (head lowered or under wing, crouching on cage floor or perch), had their eyes closed, and were immobile for ≥ 20 min. Sleep was always accompanied by robust bursting activity and persistent auditory responses in HVC. Once asleep, the birds were presented with 30–60 BOS stimulus trials of 6 s each. If the bird woke up, trials were suspended until the bird fell asleep again.

Acute recordings

A short initial surgery was performed to cement a head post to the bird's skull with dental cement (Grip Cement, Milford, DE). The bird was then allowed to recover for several days. On the day of an auditory experiment, the bird was given an intramuscular injection of 7.5 mg/kg diazepam and 0.1 ml 5% dextrose (Abbott Laboratories). Again, diazepam was chosen because it allowed easy arousal of the bird, followed in several seconds by a return to a resting state. The bird was secured in a stereotaxic apparatus by the head post and received a small injection of 2% lidocaine hydrochloride (Phoenix Pharmaceuticals) subcutaneously to the scalp. Thereafter, 1% lidocaine was applied to the scalp every 10–15 min for the duration of the experiment. To ensure normal audition and decrease discomfort, neither ear bars nor a beak bar were used. A warming pad was placed around the bird and the external temperature maintained at 34–36°C.

A window was opened in the skull over HVC and a small incision made in the dura through which a glass (5–20 M Ω) or Tungsten electrode (0.5–3 M Ω) was lowered. Sharp (World Precision Instruments, New Haven, CT) and patch (Garner Glass, Claremont, CA) glass electrodes were pulled on a micropipette puller P-97 (Sutter Instrument, Novato, CA). HVC was located by stereotaxic coordinates and by its distinctive activity patterns. For single-unit recordings, we used a loose patch technique in which a patch electrode was used to make a partial seal on an individual neuron without breaking in. This technique provides extremely stable single-unit recordings with a large signal-to-noise ratio. In some experiments, an additional window was opened in the skull and a second electrode was placed in Field L2a. Once the electrodes were in place, a series of auditory trials using the BOS and REV stimuli was run to confirm song-selective HVC auditory responses.

Prior to the beginning of each acute recording experiment, we assessed the effectiveness of an air puff to the chest in arousing the bird. The air puff was administered by depressing a bulb outside the sound attenuation chamber. The timing of each air puff was measured with a piezoresistive pressure transducer (Fujikura America, Marietta, GA) connected to an air pressure monitor (HEC 200P, Hector Engineering, Ellettsville, IN). Each air puff lasted ~300 ms. A bird was considered aroused by the air puff if we observed behavioral indicators such as the eyes opening for several seconds, feathers ruffling, and tail movements. Behavioral indicators of arousal lasted 3–10 s. When the air puff was confirmed to consistently arouse the bird, the stereotaxic apparatus was moved into the sound-attenuating chamber and the door was closed.

To control for nonarousal responses to the air puff, such as an auditory response to the puff itself, we performed several experiments in which no auditory stimulus was presented and the bird was aroused at randomly selected times during the recording session. These experiments confirmed the absence of any HVC auditory response to the air puff.

Arousal experiments were composed of 30–60 auditory stimulus trials. Each trial consisted of 6 s of baseline recording followed by ~2

s of auditory stimulation (2 song motifs) and four more seconds of baseline. All auditory stimuli were examples of the bird's song recorded in a sound attenuation chamber and digitized at 40 kHz. The stimuli were played at 50–70 dB (SPL) through an HLS410 speaker (JBL, Northridge, CA) in the sound chamber via a D-45 amplifier (Crown International, Elkhart, IN). The time interval between trials was varied during each experiment and ranged from 5 s to 3 min. On randomly interleaved trials, the bird was aroused by a puff of air to the chest 1 s before the onset of the auditory stimulus (see Fig. 9A).

Neural data from the acute experiments were acquired with an HS4 headstage (Tucker-Davis Technologies), amplified and bandpassed between 500 Hz and 10 kHz with a Brownlee Precision Model 440 instrumentation amplifier (Brownlee, Santa Clara, CA) and digitized at 20 kHz with a 100-MHz, 16-bit DAQ board (National Instruments). Acquisition software was written in Labview (National Instruments) by A. Leonardo.

Histology

After chronically implanted birds had been recorded for 2–10 days, they were deeply anesthetized with 0.1 ml 50 mg/ml pentobarbital sodium (Nembutal; Abbott Laboratories) and transcardially perfused with 0.9% saline and 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose and sectioned at 50 μ m on a freezing microtome. Electrode placement was confirmed using cresyl violet staining.

Data analysis

Data were analyzed using Matlab (The Mathworks, Natick, MA) routines written by J. A. Cardin and M. F. Schmidt. Spike events in the multiunit data from chronic experiments and multi- and single-unit data from acute experiments were measured by using a peak-detection algorithm. For each data set, the threshold was visually positioned at a point clearly above background noise but low enough to detect all observed spike events. Peristimulus time histograms (PSTHs) were calculated by binning spike events (bin size = 10 ms) during each trial and summing the resulting raster plots over 15–30 auditory trials (see Fig. 4). Preliminary analysis using bin widths of 10, 15, and 20 ms indicated that bin size did not affect the results of the data analysis described in the following text.

We observed that auditory responses in HVC, particularly during wakefulness, characteristically involved an increase in mean firing rate, a change in the temporal distribution of spike events, or both. We therefore elected to determine the presence or absence of awake auditory responses according to two separate methods. Several variations of a standard response strength method, referred to here as *method A*, have been used previously in other studies to characterize auditory responses in the song system (Doupe 1997; Janata and Margoliash 1999; Rauske et al. 2003; Schmidt and Konishi 1998; Solis and Doupe 1997, 1999). *Method A* compares the mean firing rate during the stimulus to that during the baseline on a trial-by-trial basis. This method indicates whether there is a consistent difference between the stimulus and baseline periods over the course of the auditory trials. The second method, *method B*, utilizes the summed data from a set of auditory trials to quantify the difference in both the number and distribution of spike events during baseline and stimulus periods. This method assesses whether the summed response from all the auditory trials is different from the summed baseline activity.

Method A

For each auditory trial in a set, both the mean firing rate during the auditory stimulus (FR_{BOS}) and the mean firing rate during a baseline period (FR_{BASE}) were calculated. Because the songs of individual birds varied in duration, the length of the baseline period was made equal to that of the BOS stimulus in each experiment. All baseline

periods were taken from the several seconds of recording prior to the auditory stimulus. Thus for a set of 30 auditory trials, there would be 30 pairs of FR_{BOS} and FR_{BASE} measurements. A significant auditory response was defined as a set of auditory trials where the FR_{BOS} measurements were significantly different from the FR_{BASE} measurements as assessed by a paired *t*-test.

Method B

Because auditory responses in awake birds were frequently observed to involve changes in mean firing rate, changes in the temporal distribution of spike events, or both, we used a set of criteria based on both response strength and variance measurements taken from PSTHs. For instance, 30 trials of a BOS stimulus lasting 2 s would result in a PSTH of 200 10-ms bins representing the stimulus period; the 200 bins preceding the auditory stimulus would represent the baseline period. The number of spikes in each bin was defined as the bin total. Thus we could compare the set of bin totals from the stimulus period to the set of bin totals from the baseline period. For each PSTH, we performed two calculations: 1) an unpaired *t*-test comparing the set of bin totals representing the BOS stimulus period to the set of bin totals representing a baseline period of equivalent duration and, 2) an *F* test to compare the variance of the sets of bin totals representing the same stimulus and baseline periods. An auditory response was defined as a significant difference in either comparison between baseline and stimulus periods. *Method B* thus identified three categories of auditory responses: those significant on the basis of response strength alone (RS only), those significant on the basis of variance only (VAR only), and those significant on both bases (RS&VAR). Figure 2, A and B, depicts two schematics of auditory responses in which only the magnitude or the variance of the bin totals, respectively, would be significantly different from baseline.

Response pattern

The patterns of successive auditory responses at a given recording site were compared in two ways. First, each PSTH resulting from a set of auditory trials was normalized by dividing all bin totals by the mean baseline bin total. As described in the preceding text, the baseline period preceded the BOS stimulus. Then the set of normalized PSTH bin totals from the BOS stimulus periods of the first and second sets of auditory trials were compared by a paired *t*-test. As a second test of the similarity of successive auditory responses, we also performed Pearson linear correlation analyses of pairs of PSTHs normalized as described in the preceding text. Because several pairs of responses were compared for many birds, average *r* values for each bird were calculated before comparing across behavioral states.

Index values

As noted in the preceding text, auditory responses were characterized by changes in mean firing rate, temporal distribution of spike events, or both. To quantitatively represent these characteristics in awake, sedated, and sleeping birds, we used a combination of response strength (RS) measurements, which compare mean firing rates during stimulus and baseline periods, and variance (V) measurements, which compare the distribution of spike events during stimulus and baseline periods. Variance was measured from the binned PSTH resulting from a set of auditory trials. The response strength index (RS_{INDEX}) for each auditory trial was calculated by dividing the difference between mean BOS and baseline firing rates by their sum

$$RS_{INDEX} = (FR_{BOS} - FR_{BASE}) / (FR_{BOS} + FR_{BASE})$$

RS_{INDEX} for each set of auditory trials is shown as mean $\pm$ SE. The variance index (V_{INDEX}) for each set of auditory trials was calculated as follows

$$V_{INDEX} = (V_{BOS} - V_{BASE}) / (V_{BOS} + V_{BASE})$$

V_{BOS} is the variance of the set of BOS bin totals and V_{BASE} is the variance of the set of baseline bin totals.

The advantage of the index values described in the preceding text is that they represent the entire spectrum of auditory responses within a range from -1 to 1 . An RS_{INDEX} or V_{INDEX} value of 0 indicates no difference between the BOS response and baseline activity. A positive RS_{INDEX} value indicates a greater response during the BOS presentations than during the baseline periods, whereas a negative RS_{INDEX} value indicates a smaller response during the BOS stimulus presentations than during the baseline periods. Similarly, a positive V_{INDEX} value indicates a greater variance of bin totals during the stimulus period than during the baseline period, whereas a negative V_{INDEX} value indicates lower variance of bin totals during the stimulus period than during the baseline period.

Selectivity

The selectivity of a recording site for BOS versus REV stimuli was measured using d' values (Green and Swets 1966; Janata and Margoliash 1999; Mooney 2000; Solis and Doupe 1997; Theunissen and Doupe 1998). The d' value comparing the response to BOS stimuli relative to REV stimuli was calculated as follows

$$d' = \frac{2(RS_{BOS} - RS_{REV})}{\sqrt{(\sigma_{BOS}^2 + \sigma_{REV}^2)}}$$

RS_{BOS} is the mean response strength to a set of BOS auditory stimuli. RS_{BOS} was calculated by subtracting FR_{BASE} from FR_{BOS} to give a response strength measurement for each trial and then calculating the mean RS for the set of trials. RS_{REV} is the mean response strength to the corresponding set of REV stimuli. σ_{BOS}^2 and σ_{REV}^2 represent the variance of RS_{BOS} and RS_{REV} , respectively. A d' value of 0.5 or greater was used as the criterion for a response selective for BOS over REV stimuli (Solis and Doupe 1997). Population d' values are shown as mean $\pm$ SE.

RESULTS

In this study, we assessed the presence and variability of auditory responses in HVC during wakefulness and sedation. In addition, we compared the selectivity of HVC auditory responses in awake and sedated birds. In contrast to stable, BOS-selective auditory responses observed during sedation, auditory responses during wakefulness were labile and unselective. We then used a series of carefully controlled acute and chronic recording experiments to investigate the relationship between arousal-mediated changes in behavioral state and HVC auditory responsiveness.

HVC auditory responses in awake birds

To investigate auditory processing in the avian song system during wakefulness, we recorded multiunit activity from HVC in 36 chronically implanted birds. We observed that HVC neurons in awake birds demonstrate responses to the BOS stimulus (see Fig. 4). In contrast to HVC auditory responses during sleep and anesthesia, many of the auditory responses observed in awake birds were not characterized by an increase in mean firing rate (see Fig. 2A, schematic) but by a change in the temporal distribution of spike events (see Fig. 2B, schematic).

To determine the presence or absence of auditory responses during wakefulness, each chronically implanted bird was pre-

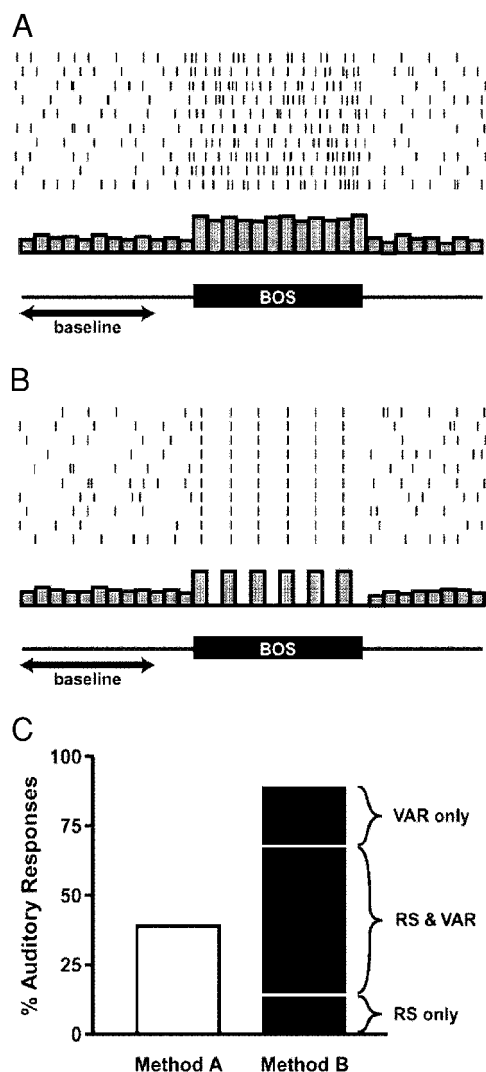


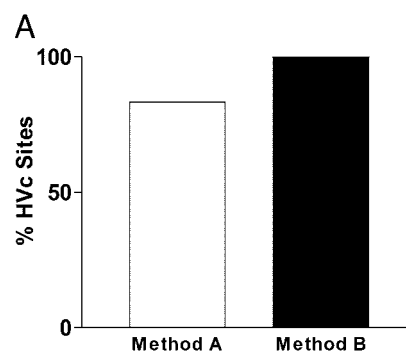
FIG. 2. HVC auditory responses during wakefulness involve changes in firing rate and temporal distribution of spike events. HVC auditory responses to the bird's own song (BOS) stimulus in awake birds were characterized by an increase in mean firing rate, a change in the temporal distribution of spike events, or both. *A* and *B*: schematic representations of auditory responses based solely on a change in mean firing rate or a change in spike event distribution, respectively. *Top*: spike raster plots; *middle*: the peristimulus time histogram (PSTH) generated from the rasters. *Bottom*: the baseline and BOS stimulus periods. Two methods were used to evaluate the presence of auditory responses. *Method A* compares mean HVC firing rates during BOS and baseline periods of each auditory trial. *Method B* uses the summed data from a set of auditory trials to compare the response strength and variance of the populations of PSTH bins corresponding to the baseline and stimulus periods. *Method B* identifies auditory responses significantly different from baseline activity in response strength, variance, or both. The example shown in *B* would be classified as an auditory response by *method B* but not *method A*. *C*: breakdown of significant HVC auditory responses of 36 birds to 136 sets of BOS stimuli using either *method A* or *B*. The *method B* column is divided into responses that were significant for the PSTH-based test of response strength alone (*bottom*), response strength and variance (*middle*), and variance alone (*top*). Using *method A*, 38.2% of auditory stimulus sets evoked a significant auditory response (*left*). In contrast, 86.0% of auditory stimulus sets evoked a significant auditory response when analyzed with *method B* (*right*).

sented with 2–12 sets of BOS stimuli over the course of 1–3 days. In total, 36 birds were presented with 136 sets of BOS stimuli. We assessed the presence of auditory responses to each set of BOS stimuli by two methods. In *method A*, the mean

HVC firing rate during the BOS stimulus was compared with the mean firing rate during baseline activity (see METHODS for details). Using this method, 38.2% (52/136) of the sets of auditory trials elicited a significant auditory response (Fig. 2C, *left*). In *method B*, we used the PSTH generated from each set of auditory trials to assess both response strength and variance (see METHODS for details). Figure 2C (*right*) illustrates the proportion of auditory responses that were significantly different from baseline in response strength, variance, or both. Using *method B*, 86.0% (117/136) of the sets of auditory trials elicited a significant auditory response. Many awake auditory responses characterized by a change in the temporal distribution of spike events were captured by *method B* but not *method A*.

We next looked at the presence of auditory responses in each individual bird during wakefulness. Figure 3A shows the percentage of multiunit HVC recording sites that demonstrated at least one auditory response during the recording period. Using *method A*, 83.3% (30/36) of the recording sites demonstrated at least one awake auditory response. Using *method B*, 100% (36/36) of the recording sites demonstrated at least one awake auditory response. Figure 3B shows the percentage of HVC recording sites that always demonstrated an auditory response

At Least One Awake Auditory Response



Always An Awake Auditory Response

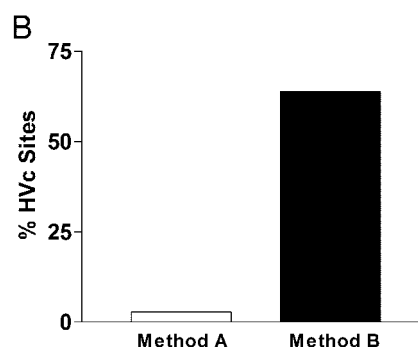


FIG. 3. Prevalence of HVC auditory responses during wakefulness. Multiple BOS stimulus sets were presented to each awake bird and the resulting auditory responses were measured using both *methods A* and *B*. *A*: number of HVC sites demonstrating at least one auditory response. Using *method A*, 83.3% (30/36) of the HVC recording sites demonstrated at least one significant auditory response. However, 100% (36/36) of the recording sites demonstrated ≥ 1 significant auditory response using *method B*. *B*: number of HVC sites always demonstrating an auditory response. *Method A* showed that 2.8% (1/36) of the recording sites always demonstrated a significant auditory response. In contrast, *method B* showed that 63.9% (23/36) always showed a significant auditory response.

during wakefulness. Although only 2.8% (1/36) of the recording sites always demonstrated a significant auditory response during wakefulness using *method A*, 63.9% (23/36) of the sites did using *method B*. These results suggest that HVC auditory responses during wakefulness are not as rare as previously thought (Dave et al. 1998; Nick and Konishi 2001; Rauske et al. 2003; Schmidt and Konishi 1998). Taken together, the data in Figs. 2 and 3 suggest not only that HVC demonstrates significant auditory responses during wakefulness but also that *method B* is more effective than *method A* in accurately representing the full range of observed responses.

Dynamic changes in HVC auditory responses during wakefulness

To assess the stability of auditory responsiveness in awake birds, we presented 30 chronically implanted birds with two to six sets of 30 BOS stimuli at different times on a single day. The mean interval between sets of auditory stimuli was 89.2 ± 18.5 min. HVC auditory responsiveness varied over the course of a single day. This variability was not due to variations in the stimulus as the set of auditory stimuli was identical throughout

each experiment. Figure 4 shows data from two awake birds that were each presented with two sets of auditory stimulus trials separated by 10 min. *Bird ZF95* had a robust auditory response to the BOS stimulus during the initial set of auditory trials but a much smaller response during the second set (Fig. 4A). In contrast, *bird ZF3* initially demonstrated little auditory responsiveness but had a robust response to the second set of trials (Fig. 4B). To quantify this type of change, we compared auditory responses recorded at different times at each HVC site. We found that 96.7% ($n = 29/30$) of the birds demonstrated significant changes ($P < 0.01$; paired *t*-test) in HVC auditory response characteristics within a relatively short period of time (5–180 min). For instance, in addition to the significant change over the 10-min interval shown in Fig. 4A, *bird ZF95* also showed significant changes in auditory response over 15-, 30-, and 180-min intervals on the same day (data not shown).

To test the hypothesis that this variability is specific to wakefulness, we compared the variability of awake auditory responses to that of sedated auditory responses in several birds ($n = 5$). Each bird was presented with multiple sets of BOS stimuli during wakefulness and then immediately sedated with a small dose of diazepam and presented with additional sets. In

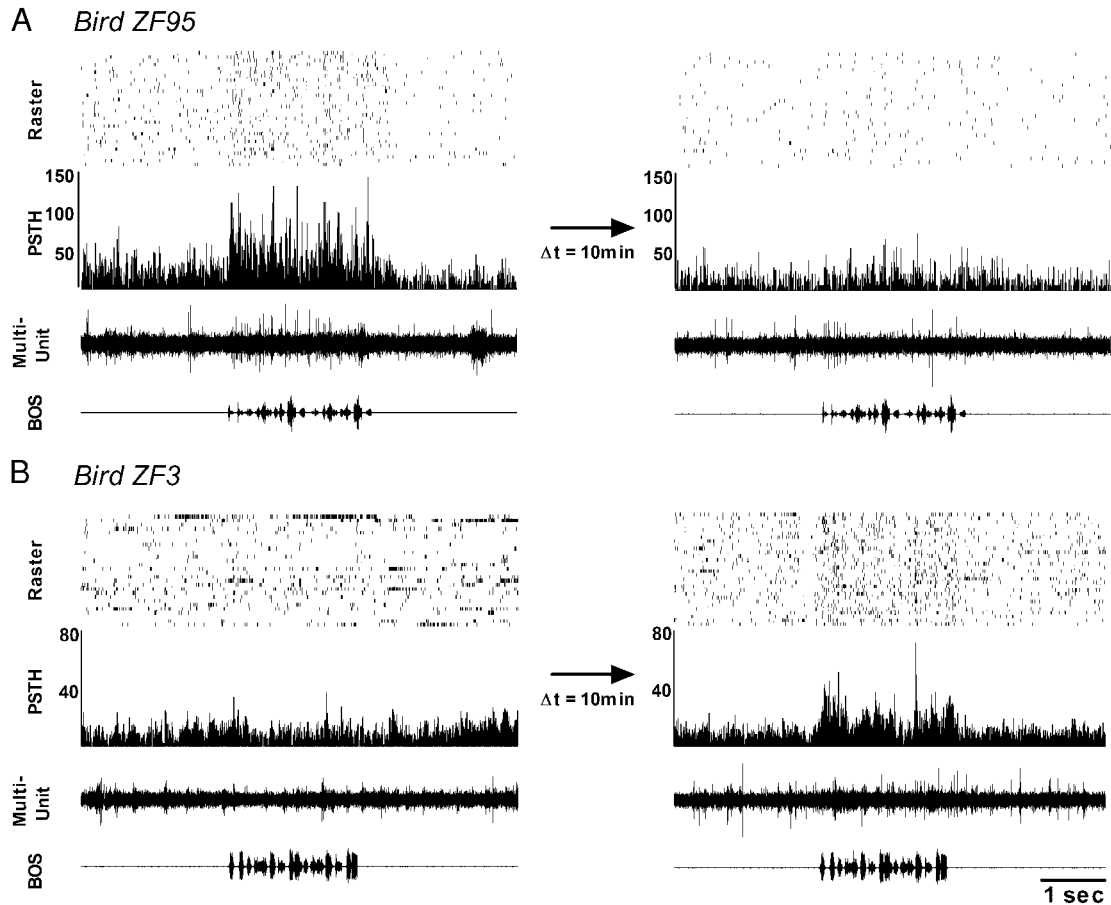
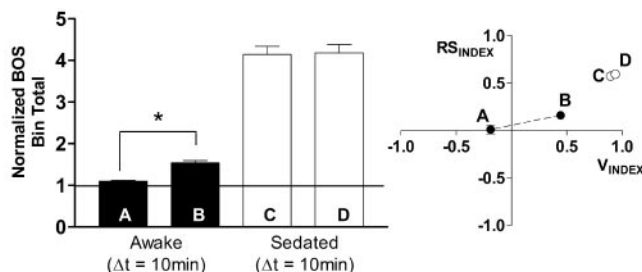


FIG. 4. HVC auditory responses during wakefulness are dynamically modulated over short time periods. HVC auditory responses vary even over short periods of wakefulness. A and B: multiunit data from 2 awake birds. In each case, the bird was presented with 2 sets of BOS stimuli separated by a 10-min interval. *Top*: raster plot representing spike events during each auditory trial. The PSTH portions were generated by summing the rasters (bin size = 10 ms). Each PSTH shows the summed neural activity in units of spike counts per bin. The traces are examples of multiunit neural activity during single auditory trials. *Bottom*: the amplitude waveform of the BOS stimulus. A: *bird ZF95* demonstrated a robust auditory response during the 1st set of auditory stimuli (*left*) but very little response to the 2nd set (*right*). B: *bird ZF3* showed little responsiveness to the 1st set of stimuli (*left*) but a large response 10 min later (*right*).

A Bird ZF178



B Bird ZF159

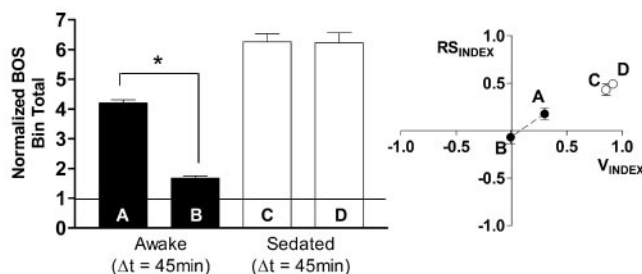


FIG. 5. HVC responses are modulated during wakefulness but stable during sedation. Results from 2 birds that were each presented with 2 sets of BOS stimuli during wakefulness and then immediately sedated and presented with 2 additional sets of BOS stimuli. These comparisons were calculated by generating a PSTH for each set of 30 auditory trials (bin size = 10 ms) and normalizing the PSTH to the baseline mean for that set of trials. The normalized PSTH bins for the 2 sets of trials were then compared by paired t -test (see METHODS). A: although bird ZF178 demonstrated a significant change in auditory response over a 10-min interval during wakefulness ($P < 0.01$), there was no change in auditory response at the same site over a 10-min interval during light sedation (NS; paired t -test). For illustration, the graph to the right shows the auditory responses at time points A–D as RS_{INDEX} and V_{INDEX} values. While there was a clear change between responses at A and B, C and D were quite similar. B: similarly, bird ZF159 showed a change in auditory response over 45 min during wakefulness ($P < 0.01$) but no change over 45 min during sedation (NS; paired t -test). Again, these auditory responses are shown at right as RS_{INDEX} and V_{INDEX} values. The responses at time points A and B were quite different, whereas the responses at C and D were very similar. —, baseline mean; *, $P < 0.01$.

all five birds, the awake auditory responses were significantly different from each other ($P < 0.01$), while the sedated responses were not (NS; paired t -test). Figure 5 shows representative examples from two birds. In Fig. 5A, bird ZF178 shows a significant change in awake auditory response over a 10-min interval ($P < 0.01$; paired t -test). In contrast, there was no change in auditory response characteristics over a 10-min interval during sedation (NS; paired t -test) or over two additional 15-min intervals during sedation (NS; data not shown). For comparison, RS_{INDEX} and V_{INDEX} values for the two awake and two sedated auditory responses are shown to the right. There was a large shift in response between time points A and B but very little change between C and D. Similarly, in Fig. 5B, bird ZF159 shows a significant change in awake auditory response over a 45-min interval ($P < 0.01$) but no change over a 45-min interval during sedation (NS; paired t -test) or three additional 10-min intervals during sedation (NS; data not shown). Again, these data are shown as RS_{INDEX} and V_{INDEX} values to the right. While there was a large change between time points A–B, C and D are quite similar. The results of the preceding analysis suggest that the observed variation in HVC auditory responses is specific to wakefulness.

Linear correlation analyses of auditory response structure were performed as an additional characterization of the variability of auditory responses during states of wakefulness and sedation. The PSTH from each set of auditory trials was normalized to the baseline mean for that set of trials. Again, only pairs of auditory responses occurring within a relatively short period (5–180 min) were compared. Successive PSTHs were then compared. Figure 6, A and B, shows examples of two awake and two sedated normalized auditory response PSTHs from one bird. Auditory responses during wakefulness show clear differences, but sedated responses are quite similar. Figure 6C shows the correlation coefficients for both awake and sedated auditory responses (Pearson correlation). The mean r value for pairs of awake responses (0.27 ± 0.02 ; $n = 69$ comparisons in 30 birds) was significantly smaller than for pairs of sedated responses (0.75 ± 0.04 ; $n = 8$ comparisons in 5 birds; $P < 0.01$; Mann-Whitney U test). The mean interval between sets of awake auditory trials was 54.2 ± 16.9 min, and the mean interval between sets of sedated auditory trials was 43.7 ± 5.2 min. These results indicate that the temporal characteristics of HVC auditory responses are quite stereotyped from moment to moment during sedation and more variable during wakefulness.

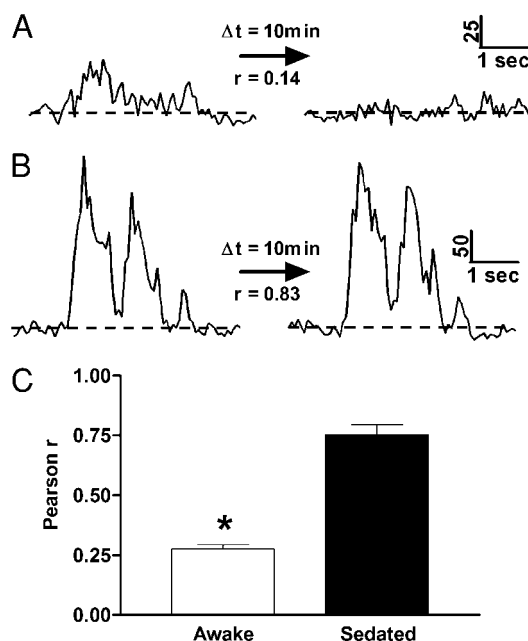


FIG. 6. Auditory response patterns are variable during wakefulness and stable during sedation. Comparisons of HVC responses to successive sets of BOS stimuli during wakefulness or sedation. Each PSTH was normalized to the baseline mean and the 2 PSTHs were then compared by Pearson correlation. A: 2 successive auditory responses from bird ZF159. Left: auditory response PSTH (bin size = 50 ms) from the 1st set of auditory trials; right: a PSTH from the 2nd set. Each PSTH shows the summed neural activity in units of spike counts per bin. B: 2 successive auditory responses from bird ZF159 during the immediately subsequent sedation period. Left: an auditory response PSTH from the 1st set of trials; right: data from the 2nd set. —, mean baseline levels. C: 69 pairs of successive awake auditory responses in 30 birds were compared (left). Mean r value was 0.27 ± 0.02 (Pearson correlation). Eight pairs of successive auditory responses in 5 lightly sedated birds were similarly compared (right). Mean r value was 0.75 ± 0.04 (Pearson correlation). R values for pairs of awake responses were significantly smaller than for pairs of sedated responses ($P < 0.01$; $n = 30$ awake and 5 sedated birds; Mann-Whitney U test).

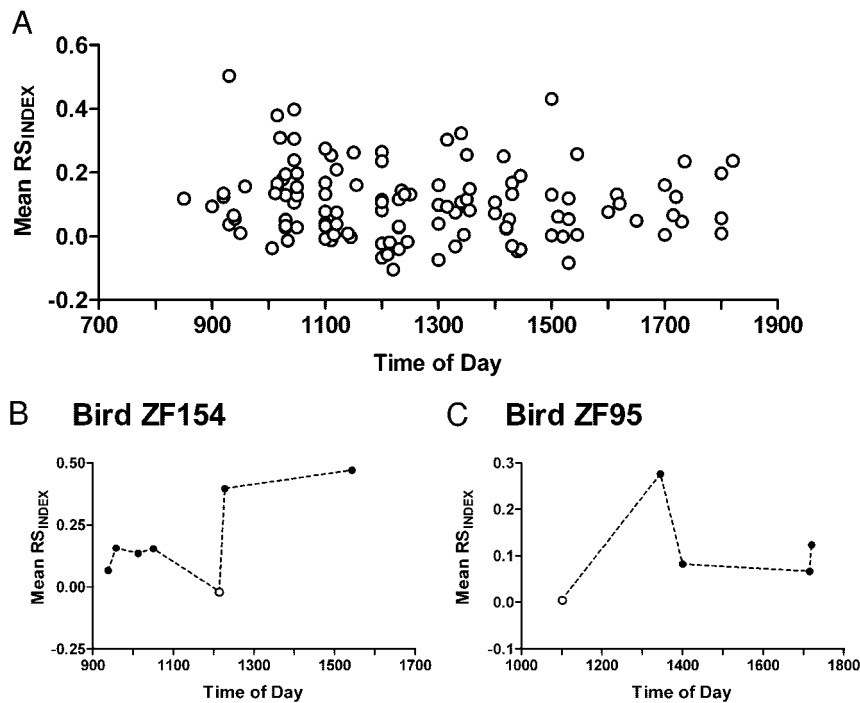


FIG. 7. HVC auditory responses are not modulated according to circadian pattern. Changes in response strength and variance of the HVC auditory responses were not correlated with time of day. **A:** response strength (RS_{INDEX}) values for 136 HVC auditory responses recorded from 36 awake birds. Each data point represents the mean HVC response to a set of 30–60 BOS stimuli. There was no significant correlation between response strength and time of day ($r = 0.01$; Pearson correlation). **B and C:** HVC auditory responses over the course of 1 day for birds ZF154 and ZF95, respectively. Each point represents the mean HVC response to 30 BOS stimuli. $\circ$, no significant auditory response; $\bullet$, a significant auditory response.

HVC auditory responses do not follow a circadian pattern

One reason for the observed variability of HVC auditory responses might be that HVC auditory responsiveness follows a circadian pattern. Figure 7A shows 136 auditory responses from 36 awake birds. There was no significant relationship between RS_{INDEX} and time of day (NS; Pearson correlation). Likewise, there was no relationship between V_{INDEX} and time of day (data not shown; NS; Pearson correlation). Individual birds did not demonstrate a circadian pattern of HVC auditory responsiveness. Figure 7, B and C, shows the auditory responses of birds ZF154 and ZF95, respectively, over the course of one day. In each case, $\bullet$ represent significant auditory responses (as determined by method B) and $\circ$ represent no auditory response.

HVC auditory responses during wakefulness are not selective for the bird's own song

A defining characteristic of HVC auditory responses during sleep and anesthesia is a strong selectivity for BOS over reversed-BOS (REV) stimuli (Lewicki and Arthur 1996; Margoliash 1986; Mooney 2000; Theunissen and Doupe 1998; Volman 1996). To test whether HVC is similarly selective for BOS stimuli during wakefulness, we presented randomly intermixed BOS and REV stimuli to freely moving, chronically implanted birds. All birds had previously shown robust, song-selective HVC auditory responses to BOS during sleep and diazepam sedation (data not shown). Comparisons were not made in cases where we observed no significant response to either stimulus. We did not observe any cases of a significant awake response to the REV stimulus in the absence of a response to the BOS stimulus. Figure 8A shows HVC auditory responses in one bird to randomly interleaved BOS and REV stimuli ($d' = -0.03$) during wakefulness. Figure 8B shows d'

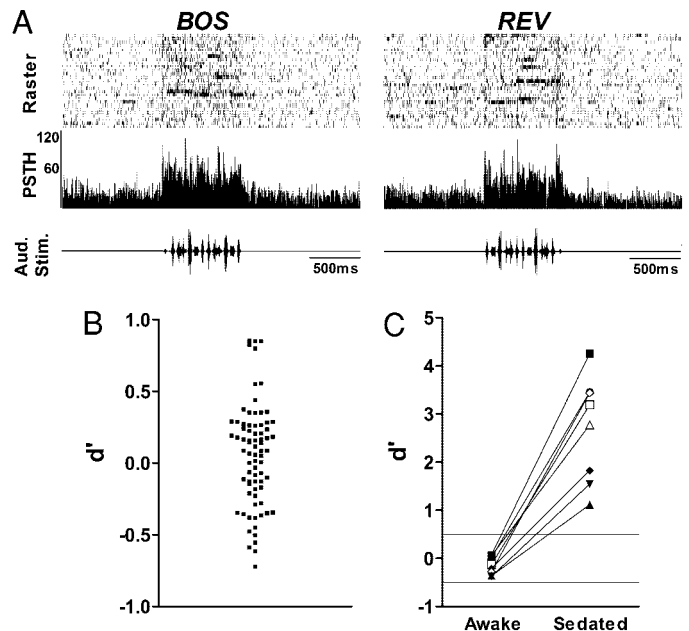


FIG. 8. HVC auditory responses during wakefulness are not song-selective. Sets of interleaved BOS and reversed-BOS (REV) stimuli were presented in random order to freely moving birds during wakefulness. **A:** response of 1 awake bird (ZF1) to randomly interleaved BOS and REV stimuli. The d' value for this pair of responses was -0.03 . **B:** responses to 76 sets of interleaved BOS and REV stimuli ($n = 30$ birds) are represented as d' values. A d' between -0.5 and 0.5 is unselective. The mean d' for awake responses was 0.05 ± 0.35 . **C:** data from 8 birds that were presented with randomly interleaved BOS and REV stimuli during wakefulness and then immediately sedated and presented with the same stimuli. All HVC sites were unselective during wakefulness (left; mean $d' = -0.15 \pm 0.06$) but became significantly more selective for BOS over REV stimuli during sedation (right; mean $d' = 2.7 \pm 0.4$; $P < 0.01$; paired t -test). Each symbol represents 1 bird.

values for 76 BOS versus REV comparisons in 30 awake birds. The mean d' for these comparisons was 0.06 ± 0.35 . While we observed a small number of selective auditory responses ($n = 12/76$), there was no overall trend toward selectivity for BOS over REV stimuli. Figure 8C depicts data from eight birds that were tested for selectivity during wakefulness and sedation on the same day. Each bird was presented with intermixed trials of BOS and REV stimuli while awake and then immediately sedated and presented with the same number of trials. Under these conditions, all HVC sites were unselective during wakefulness ($d' = -0.15 \pm 0.06$) and responded preferentially to the BOS stimulus during sedation ($d' = 2.7 \pm 0.38$; $P < 0.01$; paired t -test). These data indicate that behavioral state profoundly affects song selectivity, a fundamental characteristic of HVC auditory activity.

HVC auditory responses are tightly regulated by arousal

The highly variable nature of HVC auditory responsiveness in awake birds led us to more closely investigate the relationship between behavioral state and HVC auditory responses. To test the hypothesis that arousal modulates HVC auditory responsiveness, we used a mild sedation protocol that allowed us to record acutely in HVC while intermittently arousing the bird (see METHODS). Using this arousal paradigm during auditory stimulation, we recorded from 33 HVC sites in 24 adult zebra finches. Fifteen of these were single-unit recordings using a loose patch recording technique. During each experiment, the bird was lightly sedated, and 30–60 BOS stimuli were presented. On randomly selected trials, the bird was aroused by an air puff 1 s prior to the BOS stimulus (Fig. 9A). The two types of trials were then analyzed separately. All recording sites demonstrated a song-selective auditory response to the BOS stimulus under sedation prior to the beginning of the arousal experiments (data not shown).

All of the recording sites (33/33) demonstrated a robust suppression of HVC auditory responsiveness after arousal. Figure 9B shows data from a typical HVC multiunit arousal experiment. During the resting auditory trials (*left*), presentation of the BOS stimulus evoked a large auditory response. Arousing the bird completely eliminated the auditory response (*right*). Figure 9C shows data from a typical HVC single-unit arousal experiment. During the resting trials (*left*), the unit demonstrated an auditory response to the BOS stimulus. However, during the randomly intermixed arousal trials (*right*), the unit showed no response to the BOS stimulus.

Figure 10 shows summary data from all 33 single- and multiunit recording sites. Response strength (RS_{INDEX}) and variance (V_{INDEX}) were calculated for the resting and aroused trials at each recording site (Fig. 10A). Both response strength and variance values tended to be large and positive during resting trials and small during arousal trials. For the multiunit recording sites, mean RS_{INDEX} during resting trials was 0.34 ± 0.05 and during arousal trials was -0.07 ± 0.04 ($P < 0.01$; paired t -test; Fig. 10B1). Mean multiunit V_{INDEX} during resting trials was 0.69 ± 0.04 and during arousal trials was -0.13 ± 0.04 ($P < 0.01$; paired t -test; Fig. 10B2). For the single-unit recordings, mean RS_{INDEX} during resting trials was 0.34 ± 0.04 and during arousal trials was -0.11 ± 0.04 ($P < 0.01$; paired t -test; Fig. 10C1). The single-unit V_{INDEX} during resting

trials was 0.63 ± 0.04 and during arousal was -0.19 ± 0.06 ($P < 0.01$; paired t -test; Fig. 10C2). These results suggest a consistent suppression of both single- and multiunit HVC auditory responses by arousal.

Field L auditory responses are not modulated by behavioral state

One possible explanation for the preceding result is that arousal startled the birds and briefly interrupted all telencephalic auditory processing. Field L is a telencephalic auditory structure that receives thalamic auditory input and indirectly provides auditory input to HVC (Fortune and Margoliash 1995; Vates et al. 1996). To test whether auditory suppression on arousal was restricted to HVC and not an artifact of a startle response, we recorded simultaneously in ipsilateral HVC and Field L pairs ($n = 11$ pairs of multiunit recording sites) or in Field L alone ($n = 3$ additional recording sites) in nine birds. In all cases ($n = 14/14$), Field L exhibited robust auditory responses during both resting and aroused trials (Fig. 11A), whereas all HVC auditory responses ($n = 11/11$) were suppressed by arousal (Fig. 11B).

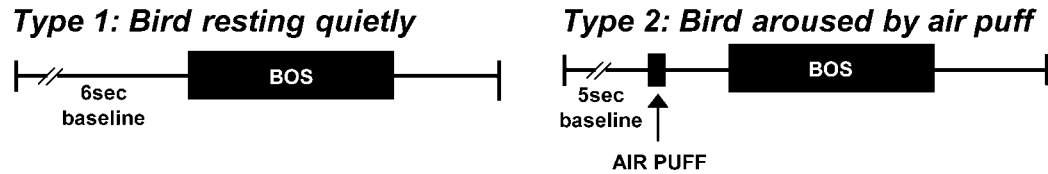
In contrast to nucleus HVC, the mean Field L RS_{INDEX} did not differ between resting (0.36 ± 0.07) and arousal (0.33 ± 0.07) trials (NS; paired t -test; Fig. 11A1). Similarly, the mean Field L V_{INDEX} was unchanged between resting (0.70 ± 0.06) and arousal (0.59 ± 0.07) trials (NS; paired t -test; Fig. 11A2). Simultaneously recorded HVC auditory responses displayed the same arousal-mediated suppression as described in the preceding text. The mean HVC RS_{INDEX} during resting trials was 0.27 ± 0.04 and during arousal was -0.22 ± 0.03 ($P < 0.01$; paired t -test; Fig. 11B1). The mean HVC V_{INDEX} during resting trials was 0.60 ± 0.05 and during arousal was -0.14 ± 0.08 ($P < 0.01$; paired t -test; Fig. 11B2). Thus while Field L auditory responses were independent of arousal, simultaneous auditory responses in the ipsilateral HVC were always significantly suppressed by arousal.

Modulation of HVC auditory responses by arousal in chronically implanted birds

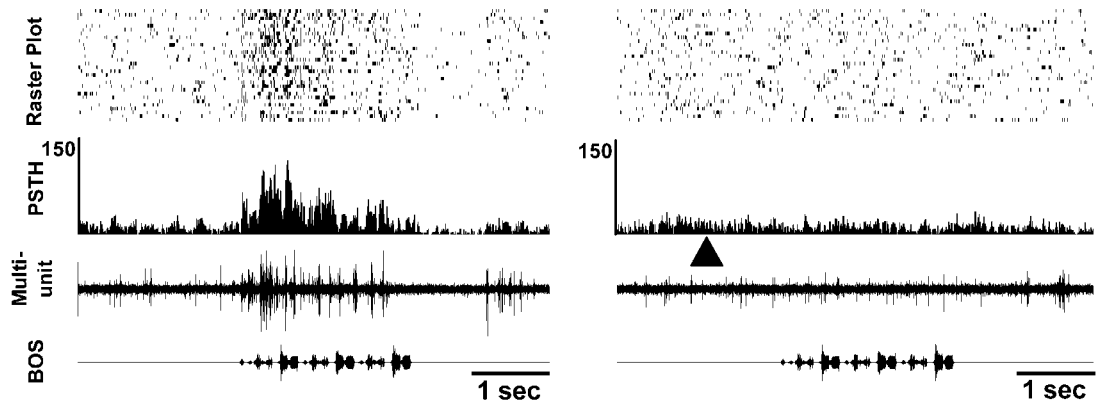
To further compare modulation of auditory responses during sedation and arousal, we recorded from HVC in chronically implanted birds using an arousal paradigm similar to that described in the preceding text. During arousal experiments, chronically implanted birds were lightly sedated and 30–60 BOS stimuli were presented. On randomly selected trials, the birds were aroused by a touch to the chest feathers (see METHODS). Arousal generally caused birds to open their eyes and move about the cage.

We tested 14 birds in this paradigm. Figure 12A shows resting and arousal auditory responses from one bird. HVC demonstrated a large auditory response during resting trials (*left*) but no response during randomly interleaved arousal trials (*right*). Figure 12, B and C, depicts auditory response values during resting and arousal trials from all birds. In all arousal experiments (14/14 birds), the auditory responses recorded during resting trials were completely suppressed by arousal. Mean HVC RS_{INDEX} was 0.37 ± 0.06 during resting trials and -0.02 ± 0.07 during arousal trials ($P < 0.01$; paired

A Experimental Protocol



B Multi-Unit Example



C Single-Unit Example

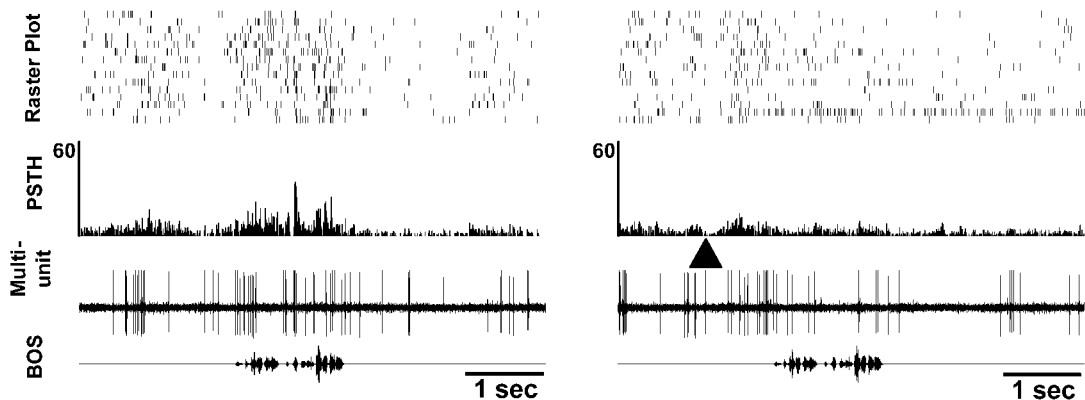


FIG. 9. Suppression of HVC auditory responsiveness by arousal. Representative data from 2 acute recording experiments showing suppression of HVC auditory responses by arousal. *A*: schematic of the 2 types of auditory trials presented in randomly intermixed order during the experiments. On resting trials, the BOS stimulus was played after a 6-s baseline period. On intermixed arousal trials, the bird received a brief air puff to the chest 1 s before the onset of the BOS stimulus. Because the trials were intermixed and the time interval between trials varied from 5 s to 3 min, the bird could not predict the onset of either the auditory stimulus or the air puff. The 2 types of trials were then separated for analysis. *B*: example of a multiunit arousal experiment in HVC. During resting trials, there was a robust HVC auditory response to the BOS stimulus (*left*). However, arousal completely eliminated the auditory response (*right*). Each PSTH shows the summed neural activity in units of spike counts per bin. *C*: example of a single-unit arousal experiment in HVC. During the resting trials (*left*), the unit responded strongly to the BOS stimulus. Arousal on randomly selected trials (*right*) suppressed the auditory response. $\blacktriangle$, timing of the air puff.

t-test; Fig. 12*B*). Similarly, mean HVC V_{INDEX} was 0.68 ± 0.06 during resting trials and -0.02 ± 0.06 during arousal trials ($P < 0.01$; paired *t*-test; Fig. 12*C*). We also performed arousal experiments on birds with chronically implanted electrodes in ipsilateral HVC and Field L ($n = 3$ birds) or Field L alone ($n = 1$ bird). As in the acute recording experiments, while HVC responses were always completely suppressed by arousal, Field L responses were unaffected by arousal (data not shown). These results, like those from the acute recordings described in the preceding text, demonstrate a significant regulation of HVC auditory responses by arousal.

Modulation of HVC auditory responses during normally occurring behavioral states

Based on our observations during the acute and chronic recording arousal experiments, we hypothesized that HVC auditory responses could be modulated by a manipulation of normal behavioral states. To test this hypothesis, we recorded from chronically implanted birds ($n = 5$) during three immediately consecutive behavioral states: *awake 1*, *asleep*, and *awake 2* (Fig. 13). The mean interval between the first *awake 1* and last *awake 2* auditory presentations was 115 ± 29.3 min.

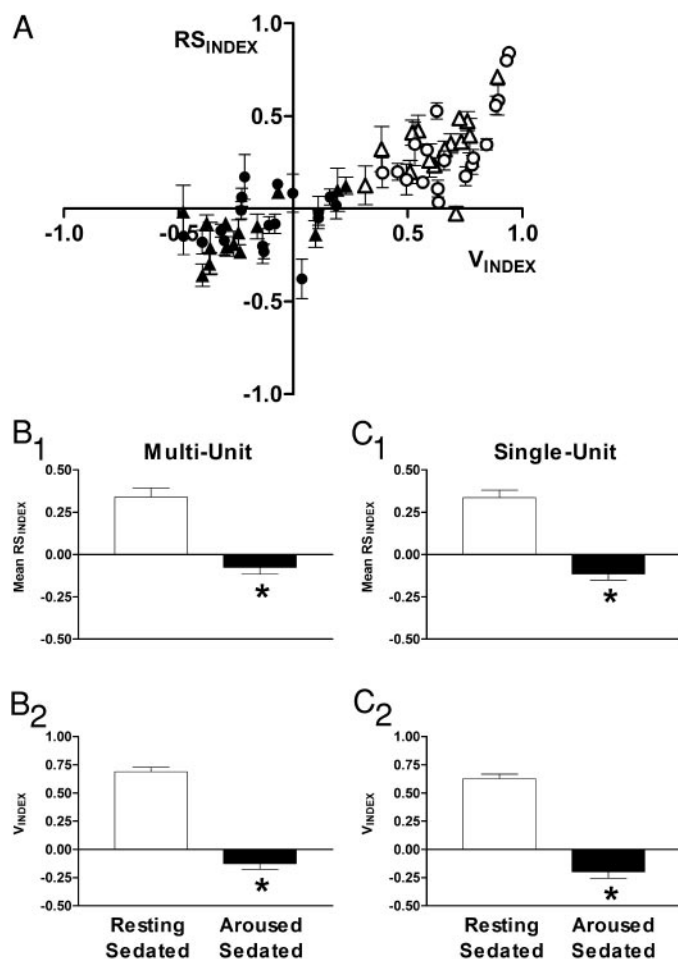


FIG. 10. Quantitative description of arousal effects on HVC auditory responsiveness. Data from all acute recording experiments. A: plot of response strength (RS_{INDEX}) and variance (V_{INDEX}) measurements from 33 HVC sites in 24 birds during resting (Δ and $\circ$) and arousal ($\blacktriangle$ and $\bullet$) trials of the acute recording experiments. Single-unit data ($n = 15$) are represented by Δ and $\blacktriangle$ and multiunit data ($n = 18$) by $\circ$ and $\bullet$. HVC response strength and variance values were large and positive during resting trials and small during arousal trials. B1: analysis of data sets from A. Mean multiunit RS_{INDEX} was significantly smaller in the aroused state than in the resting state ($P < 0.01$; paired t -test). $\square$, data from resting trials; $\blacksquare$, data from arousal trials. B2: similarly, the mean multiunit V_{INDEX} was significantly smaller during arousal than during resting trials ($P < 0.01$; paired t -test). C1: mean single-unit RS_{INDEX} was significantly smaller during arousal trials than during resting trials ($P < 0.01$; paired t -test). C2: mean single-unit V_{INDEX} was also significantly smaller during arousal trials ($P < 0.01$; paired t -test).

We first presented intermittent sets of BOS stimuli to freely moving birds until a set of stimuli evoked a significant HVC auditory response (*awake 1*). An auditory response was observed within 30 min in all birds. At the conclusion of the *awake 1* BOS auditory trials, the light in the recording chamber was turned off and the birds were allowed to fall asleep (see METHODS). During sleep, HVC auditory responses to BOS stimuli remained relatively constant in both response strength and variance values (*sleep*). The lights were then turned on and a third set of BOS auditory trials was presented as soon as the birds' eyes opened (*awake 2*).

Figure 13A shows HVC auditory responses from bird ZF97 during each of the three consecutive behavioral states. While there were large responses during the *awake 1* and *asleep* periods, HVC auditory responses to the BOS stimulus in the

awake 2 period were greatly suppressed. Figure 13, B and C, shows data from all five birds. Mean RS_{INDEX} was 0.22 ± 0.03 during the *awake 1* period and 0.01 ± 0.04 during the *awake 2* period ($P < 0.01$; paired t -test; Fig. 13B). HVC response strength and variance values did not differ between the *awake 1* and *sleep* periods (NS; paired t -test). Mean HVC V_{INDEX} was 0.45 ± 0.08 during the *awake 1* period and 0.13 ± 0.02 during the *awake 2* period ($P < 0.01$; paired t -test; Fig. 13C). The mean time over which the *awake 2* auditory stimuli were presented was 11.6 ± 1.5 min. These results demonstrate modulation of HVC auditory responses in three distinct behavioral states: *awake responsive*, with robust auditory responses, *asleep*, with robust and consistent auditory responses, and *awake suppressed*, with attenuated auditory responses.

DISCUSSION

In this study, we investigated the relationship between behavioral state and sensory processing in the song system nucleus HVC. By recording HVC population auditory responses in a large number of awake birds and comparing responses sampled over different times of the day, we show that HVC auditory responses vary from robust to undetectable levels over short periods of time. Unlike previous studies, these results suggest that HVC population responses to auditory stimuli are labile and continuously modulated by the animal's behavioral state. In contrast to auditory responses evoked during sedation, auditory responses in awake birds were generally not selective for the bird's own song. Although the presence of awake auditory responses is consistent with a role for HVC in sensory processing even when the bird is not singing, the modulation of those responses suggests nonuniformity of such sensory processing during wakefulness. Combined with our finding that arousal from light sedation or sleep can significantly attenuate HVC auditory activity in both acute and chronic recordings, our results support the hypothesis that auditory responsiveness in HVC is rapidly and reversibly shaped by the complex interplay of influences that determine the animal's behavioral state.

HVC auditory responses during wakefulness

Two primary differences may account for the disparity between the results we present here and those of earlier studies. The first difference is one of experimental methodology. In contrast to the strong, song-selective auditory responses recorded in HVC of anesthetized or sleeping animals, some studies have previously shown that auditory responses at the majority of HVC recording sites in awake zebra finches are strongly attenuated or suppressed (Nick and Konishi 2001; Schmidt and Konishi 1998). In the present study, we show that, while variable, auditory responses in HVC can be quite robust during wakefulness. Schmidt and Konishi (1998) did not observe auditory responses during wakefulness in 12/18 birds studied. As in the current study, care was taken not to handle the birds during experiments; however, recordings were performed mostly in darkness, with lights turned on only during brief periods to ensure that the birds remained awake. In the current study, all waking HVC auditory responses were observed in undisturbed birds visually confirmed to be actively moving in a brightly lit cage. Our observation that arousal suppresses HVC auditory responsiveness suggests that the gen-

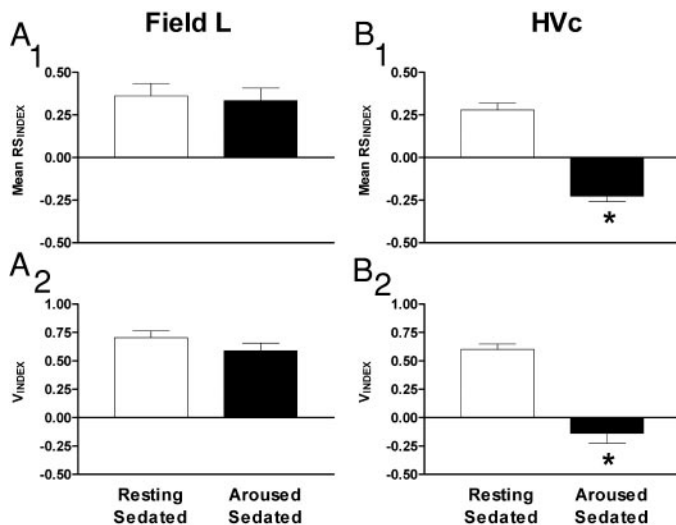


FIG. 11. Field L auditory responses are independent of arousal. Data obtained from simultaneous acute recordings of ipsilateral Field L ($n = 14$ recording sites) and HVC ($n = 11$ recording sites) during arousal experiments ($n = 9$ birds). A1: mean Field L response strength (RS_{INDEX}) was not significantly different during arousal trials (■) than resting trials (□; NS; paired t -test). A2: there was no effect of arousal on mean Field L variance measures (V_{INDEX} ; NS; paired t -test). B1: in contrast to Field L, mean HVC RS_{INDEX} was significantly attenuated by arousal ($P < 0.01$; paired t -test). B2: similarly, mean HVC V_{INDEX} was significantly decreased during arousal trials ($P < 0.01$; paired t -test).

eral lack of HVC auditory responses reported in that study likely resulted from intermittent arousal by the change in lighting.

Another study reported a similar lack of HVC auditory responses in awake birds (Nick and Konishi 2001). However, most of the awake data assessed in that study was recorded immediately after the birds were woken by external stimuli. Our observation that arousal from sleep causes a rapid suppression of all HVC auditory activity suggests that the lack of responses reported in that study may have been caused by similar arousal-mediated suppression.

A second source of differences between our results and those of previous studies is the method by which auditory responses are assessed. Previous studies have relied primarily on measurements of mean neuronal firing rate to characterize song system auditory responses both under anesthesia (Doupe 1997; Janata and Margoliash 1999; Solis and Doupe 1997, 1999) and during wakefulness (Rauske et al. 2003; Schmidt and Konishi 1998). In quantifying our data, this method, referred to here as *method A*, was sufficient to characterize a number of responses to auditory stimuli. In many cases, however, we observed that HVC auditory responses, especially during wakefulness, involved a change in the temporal distribution of spike events instead of a change in mean firing rate. This may indicate that the pattern of individual spike events, rather than mean firing rate of an entire neuronal population, encodes features within dynamic stimuli like song (for reviews, see Bair 1999; Petersen et al. 2002; Rieke et al. 1997). This observation led us to quantify the presence of auditory responses by a second method, referred to here as *method B*. This method assessed both the number and temporal distribution of spike events during auditory stimulus presentation relative to baseline activity. The combined analysis was more sensitive to the full range of possible auditory responses. The broader spectrum of

auditory responses evaluated in the present study may thus partially account for some differences between our data and those of previous studies.

Nonuniformity of auditory response properties in HVC

The relative contributions of the different types of HVC neurons to auditory responses during wakefulness are unclear. HVC contains distributed populations of RA- and Area-X-projecting neurons and interneurons (Margoliash and Fortune 1992; Nixdorf et al. 1989), which receive auditory inputs indirectly from Field L via Nif (Janata and Margoliash 1999). The precise nature of Nif targets in HVC is not known, but the three HVC cell classes have different auditory response properties in anesthetized birds (Mooney 2000). Although RA- and X-projecting neurons and interneurons all exhibit song-selective firing patterns under anesthesia, their responses are likely due to different subthreshold inputs. In particular, inhibitory inputs from song-selective HVC interneurons have been proposed to shape the auditory responses of X-projecting neurons to song stimuli (Mooney 2000).

Recording from identified single units in awake birds, Rauske et al. (2003) found that 26/38 interneurons exhibited auditory responses to the BOS stimulus during wakefulness, whereas the remaining interneurons did not. Responsive interneurons demonstrated highly stable awake auditory responses that were attenuated versions of sleeping responses by the same neurons. This stability over time was demonstrated by strong linear correlations between the PSTHs of successive auditory responses by individual interneurons. In the present study, we recorded neural activity with relatively low-impedance electrodes and therefore sampled from a large population of HVC neurons. We found low correlations between successive audi-

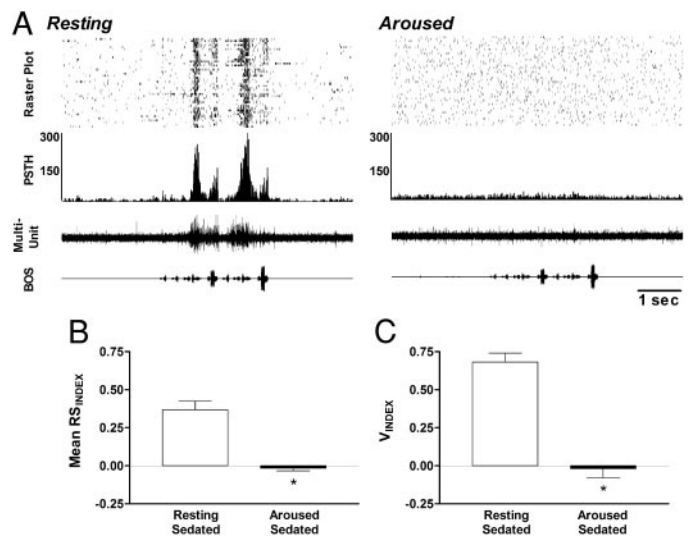


FIG. 12. Arousal modulates HVC auditory responsiveness in chronically implanted birds. A: data from 1 lightly sedated bird that was intermittently aroused during presentation of BOS stimuli. During resting trials (left), there was a large HVC auditory response. During randomly intermixed arousal trials (right), there was no auditory response. Each PSTH shows the summed neural activity in units of spike counts per bin. B: data from all HVC arousal experiments on chronically implanted birds ($n = 14$ birds); □, resting trials; ■, arousal trials. During arousal experiments, mean HVC RS_{INDEX} was significantly reduced by arousal ($P < 0.01$; paired t -test). C: similarly, mean HVC V_{INDEX} was significantly attenuated by arousal ($P < 0.01$; paired t -test).

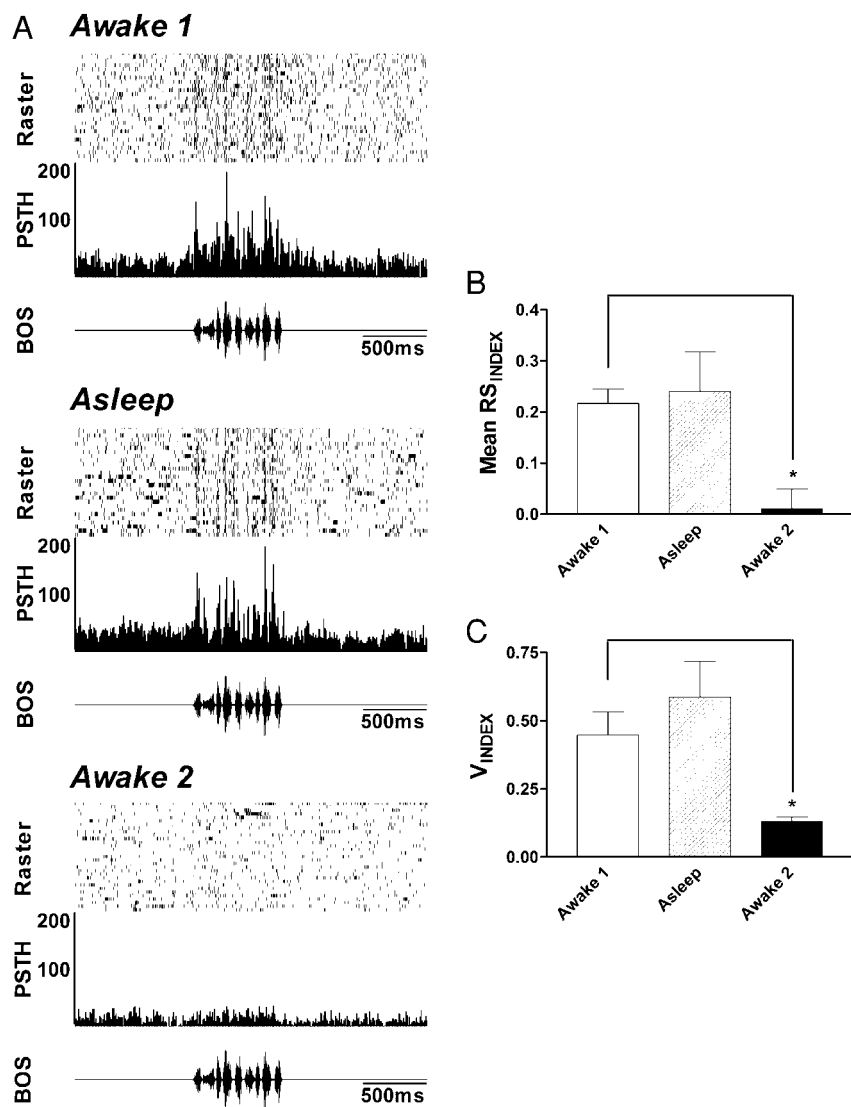


FIG. 13. Modulation of HVC auditory responsiveness by manipulation of naturally occurring behavioral states. Birds were continuously recorded in 3 consecutive behavioral states ($n = 5$ birds): *awake 1*, *asleep*, and *awake 2*. The *awake 2* period began immediately after waking from sleep. **A**: examples of auditory responses in 1 bird (ZF97) during each of the 3 behavioral states. While there was a large response during both the *awake 1* and *asleep* periods, no response was observed during the *awake 2* period. **B**: mean RS_{INDEX} during the *awake 2* period (■) was significantly smaller than during the initial *awake 1* period (□; $P < 0.01$; paired t -test). **C**: similarly, mean V_{INDEX} was significantly attenuated during the *awake 2* BOS presentations ($P < 0.01$; paired t -test). There was no significant difference in either RS_{INDEX} or V_{INDEX} between the *awake 1* and *asleep* auditory responses (NS; paired t -test).

tory responses at multiunit recording sites during wakefulness but not sedation. The difference between the stability of the interneuron responses reported by Rauske et al. and the variable responses obtained from our multiunit recordings suggests the existence in HVC of at least one class of awake responsive neurons whose auditory response properties are highly variable during wakefulness. Because RA neurons lack awake auditory responses (Dave et al. 1998), it seems unlikely that RA-projecting neurons in HVC demonstrate robust awake auditory responses. However, X-projecting HVC neurons, which provide auditory input to the anterior forebrain pathway (Doupe and Konishi 1991), may be good candidates for being responsive to auditory stimuli during wakefulness.

Although some HVC neurons may demonstrate relatively stable auditory responses during wakefulness, as indicated by Rauske et al., the results of the present study suggest that the auditory activity of a large proportion of HVC neurons can be significantly modulated by rapid changes in behavioral state. We observed repeated, complete suppression of HVC auditory responsiveness by arousal using several experimental paradigms. In addition, at 36% of our recording sites in awake birds, we periodically observed no significant response to

auditory stimuli (see Fig. 3). Assuming that our population recordings show the activity of a representative sample of HVC neurons, these results suggest the presence of a mechanism by which all auditory activity throughout HVC can be transiently eliminated.

Lack of selectivity for BOS stimuli during wakefulness

Neurons in HVC and other song system nuclei exhibit strong selectivity for BOS over REV stimuli during sleep, sedation, and several types of anesthesia (Doupe 1997; Margoliash 1983, 1986; McCasland and Konishi 1981; Mooney 2000; Vicario and Yohay 1993; unpublished data). Although we observed some isolated examples of robust selectivity for BOS (9%) or REV (7%) stimuli in HVC of awake birds, the vast majority (84%) of our recordings were unselective. In all cases, recording sites that were not selective for BOS stimuli during wakefulness showed a high degree of selectivity during sedation. Rauske et al. (2003) also found that HVC interneurons that demonstrated awake auditory responses were significantly less selective during wakefulness than during sleep. However, unlike the multiunit responses we observed, the mean awake d'

value for the interneuron responses was still significantly selective for BOS over REV (Rauske et al. 2003). While these results indicate preservation of selectivity in at least one subpopulation of HVC neurons, our data suggest that a large proportion of HVC neurons are not BOS-selective during wakefulness.

The observed lack of selectivity for the BOS stimulus in awake birds suggests that HVC may be capable of contributing to the perception of conspecific song in general. A number of behavioral studies have implicated HVC (Brenowitz 1991; Del Negro et al. 1998; Gentner et al. 2000; Okanoya and Watanabe 1995; however, see MacDougall-Shackleton et al. 1998) and other areas of the song system (Scharff et al. 1998) in auditory perception of conspecific song. Our preliminary results showing significant HVC responses to conspecific song during wakefulness support this hypothesis (unpublished observations).

Complex relationship between behavioral state and sensory responses

Studies in mammalian systems have indicated that thalamic and cortical responses to sensory stimuli are modulated by changes in a number of aspects of behavioral state, including general arousal state, motor activity, and attention (Fanselow and Nicolelis 1999; Maunsell 1995; Morrow and Casey 1992; Swadlow and Weyand 1987). These findings suggest that the general category of wakefulness includes a number of different behavioral states associated with distinct modes of sensory processing in the brain. Our observation of large changes in awake HVC auditory responsiveness over intervals as short as 5–10 min suggests rapid, fine-grained changes in sensory processing that may correlate with changing behavioral states.

To explore candidate behavioral states that may influence modulation of HVC auditory activity, we examined the effects of arousal on HVC auditory activity. We found that brief arousal from light sedation completely eliminated HVC auditory responses during both acute and chronic recordings. In addition, we manipulated the normally occurring behavioral states of chronically implanted birds and found that HVC auditory responses were suppressed on waking from a period of sleep. These results indicate that a shift to an aroused state can rapidly change HVC activity from extreme auditory responsiveness to low or completely suppressed auditory responsiveness. However, a sustained aroused state does not preclude all auditory responses, as we observed many robust auditory responses in active, alert birds. Our findings contrast with those of many mammalian studies in which a more aroused state appears to enhance, rather than suppress, sensory responses in the brain (Coenen and Vendrik 1972; Gucer 1979; Livingstone and Hubel 1981; Maffei and Rizzolatti 1965; Meulders et al. 1969; Sakakura 1968; but see Castro-Alamancos and Oldford 2002). Most mammalian studies have focused on sensory responses in the thalamus and primary sensory areas. One possible explanation for the discrepancy is that the relationship between behavioral state and sensory processing may be different in high-order sensorimotor areas such as HVC.

Local mechanisms of modulation

Field L is a primary thalamo-recipient sensory structure that is an indirect source of auditory input to HVC. In contrast to our

findings in HVC, auditory responses in Field L were unaffected by arousal in both acute and chronic recordings. In fact, Field L auditory responses in chronically implanted birds are very similar during sleep, light sedation, deep anesthesia, and wakefulness (unpublished observations). The absence of modulation of sensory responses in Field L with changes in behavioral state suggests a local modulatory mechanism within the song system.

Studies in mammalian systems have identified several neuromodulatory mechanisms affecting sensory processing modes in the brain, including activation of noradrenergic and cholinergic systems (for reviews see McCormick 1992; McCormick and Bal 1997). Similar mechanisms may also affect sensory processing in the song system. HVC receives both cholinergic and noradrenergic innervation (Appeltants et al. 2000; Bottjer 1993; Harding et al. 1998; Li et al. 1999; Mello et al. 1998; Ryan and Arnold 1981; Soha et al. 1996). Thus one possible mechanism for the modulation of HVC auditory responsiveness during wakefulness is direct neuromodulatory effects within HVC. This hypothesis is supported by evidence that injection of norepinephrine into HVC eliminates auditory responses in RA under anesthesia (Dave et al. 1998). Another possible site of neuromodulatory action is Nif, which also receives cholinergic (Ryan and Arnold 1981) and noradrenergic (Harding et al. 1998; Mello et al. 1998; Soha and Doupe 1996) innervation and is the major source of auditory input to HVC (Janata and Margoliash 1999; unpublished observations). This possibility is supported by preliminary findings indicating that auditory responses in Nif, like those in HVC, are significantly modulated by changes in behavioral state (Cardin and Schmidt 2002).

The modulation of HVC auditory responses during wakefulness is thus likely to be caused by modulation at several levels. At one level, modulation of synaptic weights within the local circuitry of HVC may change the threshold for auditory responses in the different subpopulations of neurons in this structure. At another level, modulation of activity in afferent auditory structures such as Nif may regulate auditory input to HVC and could be involved in the more dramatic suppression of auditory responses observed following arousal. This possible multistage modulation of auditory responsiveness suggests that auditory responses in the avian song system may be fine-tuned according to the bird's arousal or attentional state.

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